78. The Effect of Protein Intake on the Absorption of Calcium and Magnesium

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Lehmann & Pollak [1942*a*] observed that the phosphates and carbonates of Ca and Mg were much more soluble in solutions of α-amino-acids than they were in pure water, and it has since been shown that the solubility of commercial 'phytin' can be increased in the same way. In consequence of their observations, Lehmann & Pollak suggested that amino-acids might facilitate the absorption of Ca. To prove or disprove this hypothesis, it was decided to study in human subjects the effect of varying protein consumption upon absorption and excretion of Ca and Mg.

Subjects and experimental arrangements

There have been five subjects, four men and one woman, and each has carried out two experiments, one at a high level and one at a low level of protein intake. A low protein and a moderately low Ca diet containing fixed rations of milk, 92 % wheatmeal bread, sugar and table fat, was given in both experiments. The rest of the basal diet was made up essentially of potatoes and other vegetables, flavoured and cooked in various ways. The fare varied from day to day during the first experiment, but the menus were repeated exactly during the second. Each person weighed out and ate as much of the vegetable dishes as he or she wished during the first experiment, and adhered to the same amounts during the second.

These basal diets, which contained 45–70 g. of protein per day, were supplemented with 100–130 g. of protein (see Table 1) or with an equicalorific ration of sugar or fat. Four different sources of amino-acids were used to widen the scope of the investigation. All the protein supplements contained minerals, and, as far as possible, the intakes of Ca, Mg and P were made equal in the two experiments by administering a salt mixture with the sugar or fat supplement, or, alternatively, by reducing the milk ration when the protein intake was high. One of the protein supplements did not arrive in time to be analysed before the low protein experiment was well under way, and unfortunately in this case the Ca intakes were not equalized. The initials of the subjects, their ages, sex and weights, the duration of their experiments, the nature of their supplements and the

Table 1. *Particulars of subjects and experiments*

Initials, age and sex			Weight kg.	Duration of each exp. days	Nature and daily amount of protein supplement	Nature of non-protein supplement	Order of experiments
H.L.	31.	M.	75	6	100 g. peptone ('Difco' proteose)	Sugar	High protein (H.P.) last
J.B.	25.	M.	72	14	100 g. gelatin (Coignet's 'Extra')	Sugar	H.P. last
J.H.	32.	M.	70	14	100 g. peptone (Turner's Commercial)	Margarine	H.P. first
P.C.	26.	F.	67	14	130 g. gluten	Sugar	H.P. first
N.J.	27.	M.	67·5	14	100 g. egg-white ('Jolo' brand)	Margarine	H.P. last

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order in which they were given may be found in Table 1. H.L.'s experiments were preliminary ones; no mineral supplements were given during the low protein period; he did not collect his faeces, and used his urinary excretion of Ca and Mg as a measure of his absorption of these elements [McCance & Widdowson, 1942*c*]. Lest the increased output of urea during the high protein experiment might increase the renal excretion of Ca or Mg [Aub, Tibbetts & McLean, 1937], H.L. took 30 g. urea by mouth on each day of his low protein experiment. The peptone supplements were dissolved in water and taken with food. Gelatin was made into very stiff jellies and eaten at meal times. Some of these jellies were flavoured with fruit juice. The dry gluten was incorporated with food such as mashed potatoes. At first the egg-white powder was dusted over other foods and eaten in this way, but the raw product seemed to provoke some discomfort and diarrhoea so, during the second week of the high protein experiment, it was dissolved in warm water and cooked. The experimental organization and all the technical details closely resembled those described by McCance & Widdowson [1942*a*] and need not be given again. H.L. passed straight from his low protein to his high protein diet but all the other experiments were preceded by a fore-period of 3 days and followed by the usual after-days.

Results

Table 2 shows the intakes, absorptions and urinary excretions of Ca at the two levels of protein consumption. The term 'absorption' is used throughout this paper to mean the amount of Ca in the food minus the amount in the faeces. It is recognized that rather more than this quantity must find its way out of the intestine because the digestive

Table 2. *The effect of varying the protein intake on the absorption and urinary excretion of calcium*

All the results are expressed as mg. Ca/day.

	Low protein experiment			High protein experiment		
Subject	Intake	Absorption	Urine	Intake	Absorption	Urine
H.L.	729	—	193	660	—	320
J.H.	700	30	83	663	83	117
P.C.	598	17	94	627	106	136
N.J.	603	49	40	585	92	67
Average for J.H., P.C. and N.J.	634	32	72	625	94	107

juices secreted into the gut must contain a certain amount of Ca, all of which will not be reabsorbed. This quantity, however, will be relatively constant on a fixed diet, and it does not detract from the practical value of using the term 'absorption' in the above sense. It will be observed that the amount of Ca ingested by the subjects was of the same order in each experiment, but tended to be lower when the protein intake was high. Yet the absorptions and urinary excretions were all raised by giving the protein supplements. J.B.'s results are not given in Table 2, for although he absorbed more Ca, as the others did, in his high protein experiment, the gelatin which he consumed was contaminated with Ca, and his intakes of Ca were therefore far from equal. It is to be noted that three of the four subjects in Table 2 absorbed very little of their dietary Ca when their protein intake was low. This was partly due to their own individual idiosyncrasies, for J.B. absorbed 96 mg./day out of an intake of 640 mg./day in his low protein experiment and H.L. probably absorbed twice this amount. Further, these experiments were carried out in March, when Ca absorption tends to be at its worst in certain individuals [McCance & Widdowson, 1943], and this may be one reason for the poor absorptions.

Table 3 shows the intakes, absorptions and urinary excretions of Mg at the two levels of protein consumption. The Mg intakes were approximately the same in the high and low protein experiments, and the averaged results were almost equal. The absorptions

Table 3. *The effect of varying the protein intake on the absorption and urinary excretion of magnesium*

All the results are expressed as mg. Mg/day.

	Low protein experiment			High protein experiment		
Subject	Intake	Absorption	Urine	Intake	Absorption	Urine
H.L.	547	—	179	522	—	223
J.H.	565	179	186	500	202	183
J.B.	561	206	170	605	284	239
P.C.	438	113	97	392	126	106
N.J.	505	163	159	580	227	192
Average for J.H., J.B., P.C. and N.J.	517	165	153	519	210	180

always rose when the protein intake was increased, and the urinary excretion of four of the five subjects rose also. If expressed as a percentage of intake instead of in absolute units, the urinary excretions rose in all five. The absorptions were not doubled or trebled, as those of Ca, but this was not surprising. Mg salts are generally more soluble than the corresponding Ca salts, and Mg ions tend to be much more freely absorbed from the intestine than Ca ions. Thus, on the low protein diet these subjects absorbed an average of 5 % of their dietary Ca, but 32 % of their dietary Mg. On the high protein diets the absorptions were 15 and 41 % for Ca and Mg respectively. Each therefore was increased by about 10 % of the intake. The average absorptions of Ca rose from 1·2 to 3·5 m.eq./day and those of Mg from 13·8 to 17·5, so that increasing the protein intake actually promoted the absorption of more Mg than of Ca ions.

Discussion

It is remarkable that the relationship between protein intake and Ca absorption has not been appreciated before. Lactose and fats have been reported to influence absorption, but Aub *et al.* were so dissatisfied with the state of knowledge existing in 1937 that they set out deliberately to search for the missing factor or factors in Ca absorption. They tried the effect of urea, with negative results, and the reason for this is now clear [Lehmann & Pollak, 1942*b*]. They did not, however, try the effect of protein. As a matter of fact, at least three workers or groups of workers have had results before them which demonstrated this influence of amino-acids on calcium absorption, but none of them quite realized the significance of what they had got. Mellanby [1921] observed, in the course of his studies of canine rickets, that lean meat 'had a definite anti-rachitic effect'. He recognized that its action in this respect was of a secondary nature and not comparable with that of the anti-rachitic vitamin. He did not determine the mechanism of this adjuvant anti-rachitic action of meat, but it is reasonable now to suggest that it was due to the protein it contained. The same explanation probably covers another observation of Mellanby [1925], namely, that separated milk had some anti-rachitic effect over and above that due to the Ca it contained. It must be admitted, however, that this was a subject of controversy before 1925, and has been ever since [Henry & Kon, 1939; Kempster, Breiter, Mills, McKay, Bernds & Outhouse, 1940]. Adolph & Chen [1932] compared milk and soya bean as sources of Ca to man at two levels of protein intake. Their metabolic periods were only 4 days in length and they had only three subjects, but their figures show that Ca was absorbed more freely when the protein consumption was high, and they noted that 'increasing the protein intake facilitated the attainment of Ca equilibrium'. They do not, however, seem to have pursued the matter further, and in one respect their results are a little puzzling, for they did not find that an increased absorption was always associated with an increased urinary excretion [McCance & Widdowson, 1942*c*]. This may just be an expression of the fact that their experiments were rather slight and their periods too short for satisfactory metabolic work. Kunerth & Pittman [1939] and Pittman & Kunerth [1939] found that raising the N intake of three young women from an average of 4·01 to one of 10·92 g./day improved their Ca absorptions from 42 to 102 mg./day, and also increased the amounts excreted in the urine. The intakes of Ca averaged 458 and 436 mg./day in the two experiments so that the absorptions might have shown even more increase if the intakes had been exactly the same. Their metabolic studies were long ones and they commented on the fact that the 'high protein diet improved appreciably the utilization of ...Ca...by human subjects compared with the low protein diets of the earlier investigation'.

There have also been experiments on rats in which this effect of protein may have contributed to the results [Conner & Sherman, 1936; Conner, Kao & Sherman, 1939; Kao, Conner & Sherman, 1941], and others on pigs in which varying the protein intake was found to have no effect on mineral metabolism [Woodman, Evans & Turpitt, 1937]. These last experiments, however, were not arranged in the best possible manner for proving the point now being discussed, for the pigs were growing rapidly, Ca was probably not a factor limiting their growth, and the same animal does not seem to have been used for metabolic studies at both levels of protein intake. Hence absorptions at the two levels cannot really be compared.

Now that a relationship has been established between protein intake and Ca and Mg absorptions, the question at once arises whether P absorption is also improved, and whether Fe forms soluble and absorbable co-ordination compounds with amino-acids. Unfortunately the present studies do not provide a definite answer to either question. Of the three subjects whose Ca and P intakes were successfully equalized, the P absorptions of two (P.C. and J.H.) were improved by raising the protein intake, while that of the third (N.J.) was made slightly worse. J.B. absorbed the same amount of P in both his experiments. This suggests that protein was facilitating his P absorption, for, had it not been doing so, his absorption of P would have fallen off owing to his increased consumption of Ca from his gelatin. It seems possible, therefore, that protein does facilitate the absorption of P, but the effect is a small one.

The Fe intakes were not satisfactorily equalized in two of the subjects. The others showed no increased absorption when the protein intakes were raised, but it would be unwise to generalize from such limited material.

The recognition of the relationship between protein intake and Ca absorption clears up a number of other observations which have been made from time to time. It helps to explain why the Eskimos, who are largely carnivorous and whose Ca intakes cannot be large, tend to be well grown and have good teeth, and it provides an additional reason for recommending high protein diets in pregnancy and lactation. It makes the position of meat in the diet rather an interesting one, for this substance, admittedly a poor source of Ca, may so promote its absorption by providing a plentiful supply of amino-acids that it becomes the equivalent of a food rich in Ca [Mellanby, 1925]. Some of this effect may be neutralized by the phosphates which are present in meat and which themselves tend to inhibit the absorption of Ca [McCance & Widdowson, 1942*b*]. In H.L.'s experiments however, the large amount of phosphates in the peptone did not prevent a great increase in the absorption of Ca.

It is well known that individuals vary greatly in their ability to absorb Ca. These variations are not likely to originate as a rule in differences in protein consumption. J.B. for example, absorbed more Ca from an intake of 640 mg./day and a protein intake of 0·97 g./kg. of body weight/day than either J.H. or N.J. on similar intakes of Ca and protein intakes of the order of 2·4 g./kg./day. There is little doubt that H.L. absorbed more still, but the actual figures for his absorptions are not available.

Given that the level of protein intake is a factor in Ca absorption, the problem of assessing its quantitative significance is obviously a practical and important one. Clearly, on the one hand, it provides a method of improving an adult's absorption of Ca without increasing his Ca intake and, since vitamin D in physiological doses does not seem to have this effect, juggling with the phytates and phosphates [McCance & Widdowson, 1942*a*, *b*] or raising the protein intakes would seem to be the only certain methods of doing so. If required, moreover, the protein intake can be varied with the Ca intake so that they will have a synergic action in promoting or impeding Ca absorption. There is a further point. If Tables 2 and 4 are considered, it can be shown that increasing the protein intake of J.H., P.C. and N.J. from an average of 55 to one of 165 g./day increased the Ca absorption from 32 to 94 mg./day. One might very well argue from this that these subjects would have absorbed no Ca at all in their low protein experiment had it not been for the protein in their basal diets. These considerations show that the protein in a diet may be an absolutely vital factor in Ca absorption. This is probably not the case in Mg absorption, for reasoning similar to the above suggests that on a protein-free diet the Mg absorptions would have been of the order of 140 mg./person/day. On the other hand, the control diets in these experiments were relatively low in protein and the supplements were large. The actual figures are given in Table 4. Normal diets in adult life

Table 4. *Protein intakes during the low and high protein experiments*

Subject	Low protein diet		High protein diet	
	g./day	g./kg. body wt./day	g./day	g./kg. body wt./day
H.L.	54	0·72	154	2·1
J.H.	60	0·86	160	2·3
J.B.	70	0·97	170	2·4
P.C.	45	0·67	175	2·6
N.J.	61	0·90	·161	2·4

contain about 1 g. of protein/kg. of body weight, and it would be impracticable to double them, at any rate for long periods, even for therapeutic purposes. Consequently, varying the protein intake is unlikely in practice to bring about changes in absorption as great as those given in Tables 2 and 3. These are large, but they could probably have been achieved more easily—and much more cheaply—by increasing the mineral intakes. Thus, McCance & Widdowson [1942*a*] found that the addition of 0·1 g. Ca to 100 g. brown bread raised the average Ca absorption of five subjects, who were eating 1–1·5 lb. bread a day, from 43 to 149 mg. a day, and changing to a white bread diet produced about the same effect. This increase of 3–4 times is of the same order as that produced by 100 g. protein (Table 2).

It would seem, therefore, that while a good protein intake benefits Ca and Mg absorptions, and while it has been suggested that very little Ca would be absorbed from a protein-free diet, yet a high protein intake can never replace a satisfactory quantity of Ca itself, and should never even be considered as a substitute.

Summary

1. Metabolic studies on five healthy adults have shown that increasing the protein intake raised the amount of Ca and of Mg absorbed from the gut and subsequently excreted in the urine.

2. It is suggested that very little Ca would be absorbed if the diet contained no protein or amino-acids.

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